

Effect of Ruminant Grade Menhaden Fish Meal on Reproductive and Productive Performance of Lactating Dairy Cows¹

J. M. BURKE,² C. R. STAPLES,^{2,3} C. A. RISCO,⁴
R. L. DE LA SOTA,⁵ and W. W. THATCHER²
University of Florida, Gainesville 32611

ABSTRACT

Menhaden fish meal, fed at 0.7 kg/d [2.7% of dietary dry matter (DM)], replaced blood meal and meat and bone meal (2.0% of dietary DM) in the diet fed at dairy A (n = 341) and replaced blood, meat and bone, and corn gluten meals (3.2% of dietary DM) in the diet fed at dairy B (n = 300). Cows consumed the experimental total mixed diets from ~24 to 109 d postpartum. Cows were synchronized for estrus using injections of GnRH agonist at 51 d postpartum, PGF_{2α} 7 d later, and artificial insemination at detected estrus. Resynchronization occurred using the same program if estrus was not detected. Diet failed to alter reproductive responses at synchronized artificial insemination. Pregnancy rate at 120 d postpartum was not altered by diet at dairy A (65.4% vs. 60.2%) but was improved by dietary fish meal at dairy B (31.9% vs. 41.3%; interaction of dairy and diet). The dynamics of corpus luteum regression were altered in cows fed fish meal at dairy B. Dietary fish meal did not influence milk production of cows at dairy A but increased production of milk (2.3 kg/d) and protein (0.1 kg/d) of second parity cows at dairy B. When dietary fish meal was fed to cows at dairy B, production of milk fat and 4% FCM was higher for cows in parity 5 or greater than that for cows in fourth parity.

(**Key words:** body condition, fish meal, lactation, reproduction)

Abbreviation key: BCS = body condition score, BUN = blood urea nitrogen, CSLCFA = calcium salts of long-chain fatty acids, DHA = docosahexaenoic

acid, EPA = eicosapentaenoic acid, GnRHa = GnRH agonist, PP = postpartum.

INTRODUCTION

The supplementation of fatty acids can influence metabolic events that are important to successful reproduction of dairy cows. An emulsion of soybean oil (50% linoleic acid) infused i.v. into Holstein heifers (16) resulted in increased plasma concentrations of PGF_{2α} metabolite and altered follicular dynamics. In addition, the infusion of soybean oil increased numbers of ovarian follicles and the diameter of the largest follicle. In another study (17), calcium salts of long-chain fatty acids (CSLCFA) fed to lactating dairy cows increased the numbers of 3- to 5-mm follicles, the numbers of follicles >15 mm in diameter, and the size of the preovulatory follicle of a synchronized estrous cycle during the early postpartum period. The increased size of the preovulatory follicle caused by CSLCFA was found to be the result of the fatty acids themselves rather than a result of an improved energy status of the cows (15). In addition, by 120 d postpartum (PP), dietary CSLCFA fed at the rate of approximately 0.5 kg/d improved conception rates of lactating Holstein cows from 52 to 86% (12).

Supplemental fat can influence uterine metabolism, as well as metabolism of the ovary. The peak plasma concentration of PGF_{2α} metabolite in response to a pulse-dose of oxytocin administered i.v. on d 15 of the estrous cycle was depressed in lactating cows that received an abomasal infusion of 0.45 kg/d of yellow grease (17% linoleic acid) relative to those cows infused with water, glucose, or tallow (2% linoleic acid) (21). The dominant follicle of cows that received yellow grease grew more rapidly than did follicles of cows that received tallow.

Fish meal contains approximately 8% fat, of which two-thirds is long-chain, polyunsaturated fatty acids, including the unique fatty acids eicosapentaenoic (C_{20:5n-3}; EPA) and docosahexaenoic (C_{22:6n-3}; DHA). Typically, dietary unsaturated fatty acids are biohydrogenated by ruminal microorganisms. However, the EPA and DHA that are found in fish oil

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²Department of Dairy and Poultry Sciences.

³To whom correspondence should be addressed.

⁴Department of Large Animal Clinical Sciences.

⁵Current address: Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de la Plata, Calle 60 y 118, 1900 La Plata, Argentina.

appear to escape biohydrogenation (2, 23, 30). Therefore, dietary fish meal may result in the metabolic uptake of these fatty acids by reproductive tissues of the lactating cow. These fatty acids can inhibit cyclooxygenase activity and, in turn, can decrease PGF_{2 α} synthesis (29).

Fish meal in the diets of lactating dairy cows has improved conception rates, although the mechanisms of action have not been elucidated. Armstrong et al. (1) reported a 20 percentage unit increase in conception rates by partial replacement of soybean meal with fish meal in the diet. Bruckental et al. (5) reported a 20 percentage unit increase in pregnancy rate when fish meal rather than soybean meal was supplemented to the diet. Whether these responses were due to a reduction in intake of ruminally degradable protein, or an increase in intake of long-chain polyunsaturated fatty acids, or both is unknown.

Of the available feedstuffs that are high in undegradable protein, fish meal often is effective in improving milk production. In a summary of eight studies in which corn silage was the main dietary source of forage (24), fish meal increased milk production by a mean of 1.6 kg/d per cow. Milk protein content tended to be increased or unchanged, and milk fat content tended to be decreased or unchanged. Decreases in milk fat content were more common when the amount of fish meal fed was high (1 to 2.6 kg/d). When ruminal pH was <6.0, excess intake of fish oil depressed fiber digestion via toxicity to ruminal microbes, which may account for the lowered fat content of milk (13). Less than 0.75 kg/d of dietary fish meal is not likely to depress milk fat content. In addition, fish meal provides greater concentrations of the essential and often limiting amino acids, lysine and methionine, than do other typical concentrated protein feedstuffs.

The objective of this study was to determine the effect of dietary fish meal (EPA and DHA) on reproductive performance, milk production and composition, body condition score (**BCS**), and blood urea nitrogen (**BUN**) of Holstein cows at two dairy farms in Florida.

MATERIALS AND METHODS

Dairy A

The experiment was conducted on a Florida dairy herd from December 1994 to May 1995 using 341 multiparous lactating Holstein cows. Cows were housed in an open-sided, free-stall barn with a concrete floor and self-locking stanchions. Sand was used for bedding. Cows were assigned randomly to a control diet (n = 166), which contained blood meal plus meat and bone meal as ruminally undegradable pro-

tein sources, or to the experimental diet, which contained a ruminant grade Menhaden fish meal (n = 175; Sea-Lac[®]; Zapata Haynie Corp., Hammond, LA). Composition of the diets is presented in Table 1. Targeted intake of fish meal was 0.7 kg/d per cow. Cows were fed the diets starting at 25 ± 0.5 d PP and continued on the same diet for a mean of 88 ± 2 d (~113 d PP). Samples of the total mixed diet were collected weekly, dried at 55°C, composited monthly, and analyzed for CP, soluble CP, ether extract, NDF, ADF, calcium, phosphorus, potassium, and mag-

TABLE 1. Ingredient and chemical composition of experimental diets.

Composition	Dairy A		Dairy B	
	Control	Fish meal	Control	Fish meal
Ingredient, % of DM				
Corn silage	21.5	21.5	22.4	22.4
Alfalfa hay	7.2	7.2	5.3	5.3
Cottonseed hulls	5.7	5.7
Bermudagrass hay	4.7	4.7
Hominy	31.0	31.0	30.9	30.5
Whole cottonseed	12.6	12.6	9.0	9.0
Wet brewers grains	5.4	5.4	11.0	11.0
Lacto-Whey ¹	7.0	7.0
Fish meal	...	2.7	...	2.8
Soybean meal	6.7	6.7	1.3	2.4
Meat and bone	1.0	...	0.7	...
Blood meal	1.0	...	1.0	...
Corn gluten meal	1.5	...
Mineral and vitamin premix	8.9 ²	8.2 ²	4.2 ³	3.8 ⁴
Chemical				
DM, %	58.1	58.1	52.9	52.9
NE _L , Mcal/kg of DM	1.71	1.71	1.71	1.71
CP, % of DM	18.1	18.1	19.8	19.5
Soluble protein, % of CP	25.4	24.6	35.2	33.7
RUP, % of DM	7.0	7.1	6.9	6.9
Ether extract, % of DM	5.6	5.7	6.7	6.7
NDF, % of DM	28.9	28.8	35.8	35.1
ADF, % of DM	18.3	18.3	18.9	18.4
Ca, % of DM	0.98	1.01	1.05	1.00
P, % of DM	0.52	0.57	0.52	0.52
K, % of DM	1.4	1.4	1.6	1.5
Mg, % of DM	0.37	0.34	0.37	0.38

¹Packerland Whey Products, Inc. (Luxemburg, WI).

²Premix contained 39.2% soybean hulls, 16.0% CaCO₃, 9.7% NaHCO₃, 7.3% KCl, 7.3% tallow, 5.1% wheat middlings, 3.6% urea, 3.2% MgKSO₄, 2.5% MgO, 2.5% CaHPO₄, 2.4% NaCl, 1.0% of a trace mineral and vitamin premix (Purina Mills, Mulberry, FL), and 0.2% Zinpro 100 (Zinpro Corp., Chaska, MN)..

³Premix contained 24.6% sodium sesquicarbonate, 23.8% CaCO₃, 18.6% MgKSO₄, 17.2% KCl, 6.1% yeast, 5.4% CaHPO₄, 1.8% of a trace mineral and vitamin mix (Purina Mills), 1.7% MgO, and 0.8% niacin.

⁴Premix contained 26.8% sodium sesquicarbonate, 25.0% CaCO₃, 18.3% KCl, 18.0% MgKSO₄, 6.7% yeast, 2.4% MgO, 1.9% of a trace mineral and vitamin mix (Purina Mills), and 0.9% niacin.

nesium (Northeast DHIA Forage Testing Laboratory, Ithaca, NY). Orts were weighed daily, and daily DMI for each group of cows were calculated.

Cows were milked three times daily. Milk production was measured at one milking per month, and daily milk production was calculated by the DHIA. The maximum number of milk production estimates per cow was five. Composition of milk was not measured.

The voluntary waiting period for breeding was 60 d PP. At 30 ± 3 d PP, cows were injected i.m. with PGF_{2 α} (25 mg of Lutalyse[®]; Pharmacia and Upjohn, Inc., Kalamazoo, MI) to regress any existing corpus luteum and potentially to increase the number of estrous cycles prior to first AI. An additional benefit of the injection of PGF_{2 α} is the subsequent dilation of the cervix, which is associated with estrus and contributes to the optimization of the uterine environment by reducing the occurrence of metritis or pyometra (25). Cows were synchronized for estrus with an i.m. injection of GnRH agonist (**GnRHa**; 8 μ g of Buserelin; Hoechst-Roussel Agri-Vet, Somerville, NJ) at 51 ± 3 d PP, followed 7 d later with an injection of PGF_{2 α} (Table 2) (4, 27, 33). This program synchronized both follicular development and regression of the corpus luteum (18, 31). The methods of estrus detection used were visual observation of cows for estrus throughout the day, use of Kamar heat mount detectors (Kamar Marketing Group, Portland, ME), and visualization of tailheads that were chalked (All-Weather[®] Paintstik[®]; LA-CO Industries, Inc./Markal Co., Chicago, IL). Cows were inseminated with frozen-thawed semen within 12 h of detected estrus by one of five technicians. Thirty-

seven bulls were used in the trial. Semen source used was randomly distributed across both treatments. Cows were considered responders to first synchronization if signs of estrus were displayed within 7 d of injection of PGF_{2 α} . Cows that did not show signs of estrus at the first synchrony were resynchronized 7 d later by an injection of GnRHa at approximately 65 d PP followed by an injection of PGF_{2 α} 7 d later and then were bred to detected estrus. Any cows that did not exhibit estrus after two synchronization attempts were allowed to undergo spontaneous cycles and were bred upon observation of standing estrus. Cows that were bred at synchrony but did not conceive were rebred after they returned to estrus. Palpation of the uterus and its contents per rectum at ≥ 42 d post-AI was used to diagnose pregnancy. Pregnancy rate was defined as the proportion of synchronized cows that were pregnant. Conception rate was defined as the proportion of cows that were detected in estrus and inseminated that were pregnant.

Dairy B

The experiment was conducted on a Florida dairy herd from January to June 1995 using 300 multiparous lactating Holstein cows. Cows were housed in an opened-sided barn with a concrete floor. Old hay and recycled newspapers were used as bedding. In addition, cows had 24-h access to a sandlot and cooling pond. Control cows (146 cows with even-numbered ear tags) were assigned to a diet containing a mixture of four ruminally undegradable protein feedstuffs (Table 1). Cows with odd-numbered ear tags were assigned to the fish meal diet ($n = 154$; Table 1). Feed samples were collected and analyzed chemically as described previously. Cows were fed diets starting at 23 ± 5 d PP and continued on the same diet for 82 ± 2 d (~ 105 d PP).

Cows were milked three times daily. Milk production was measured daily and averaged weekly using the Afimilk[®] system (S.A.E Afikim, Kibbutz Afikim, Israel) for a maximum of 17 wk per cow. Milk was measured biweekly for fat and protein content. The SCC were measured monthly.

The reproductive management program for the cows that were synchronized during the first 8 wk of the study (strategy 1; $n = 225$) was similar to that used at dairy A. A timed AI program was utilized for the cows that were synchronized during the last 6 wk (strategy 2; $n = 75$). This program change was implemented because of a low detection rate of estrus at first synchrony (50%; Table 3). Previous research from our laboratory indicated that pregnancy and conception rates using timed AI in primiparous and multiparous cows were comparable with rates obtained using the program followed in strategy 1 (6).

TABLE 2. Reproductive program for dairy A (only strategy 1 was used) and dairy B (strategy 1 was used for 225 cows, and strategy 2 was used for 75 cows).

	Strategy 1		Strategy 2	
	Injection	Time	Injection	Time
30 ± 3 d PP ²	PGF _{2α}	1200 h	PGF _{2α}	1200 h
51 ± 3 d PP	GnRHa ³	1200 h	GnRHa	1200 h
58 d PP	PGF _{2α}	1200 h	PGF _{2α}	1200 h
60 d PP			GnRHa	1200 h
61 d PP	AI at Estrus		Timed AI	0600 h
65 ⁴ d PP	GnRHa	1200 h		
72 d PP	PGF _{2α}	1200 h		
	AI at Estrus			

¹Represents a range of days.

²Postpartum.

³GnRH agonist.

⁴If a cow was not detected in estrus, then resynchronization occurred at 65 d PP for cows in strategy 1.

The timed AI program required an additional injection of GnRHa 48 h after the PGF_{2α} injection administered at 58 d PP, and cows were inseminated 16 h later without regard to estrus detection. For strategy 2, a second synchronization was not necessary because all cows were inseminated. The method of estrus detection was a combination of visual observation and pedometer readings using the Afimilk[®] system. Inseminations (using a total of 80 bulls) were performed by 1 of 12 technicians at approximately 8 h after the activity (steps per hour) of the cow reached two standard deviations above individual mean activity. Semen sources used were randomly distributed across treatments. Pregnancy diagnosis per rectal palpation occurred ≥45 d post-AI.

Determination of BCS, Progesterone, and BUN

Blood samples were collected from the coccygeal vessel immediately prior to injections of GnRHa at 51 and 65 d PP and injections of PGF_{2α} at 58 and 72 d PP. For cows managed using timed AI, samples were collected from 56 cows at dairy B before the second injection of GnRHa at 60 d PP to estimate the proportion of cows that responded to injection of PGF_{2α} at 58 d PP. Samples were held constantly in ice and centrifuged (3000 × *g* for 20 min at 4°C) within 16 h of collection before plasma was collected. Plasma was stored at -20°C until analyzed for progesterone (14) and BUN (20). Interassay and intraassay coefficients of variation for the progesterone assay were 9.3 and

TABLE 3. Effect of dietary fish meal on rates of estrus detection, pregnancy, and conception and other reproductive responses of lactating cows at two Florida dairy farms. Least squares means are adjusted for appropriate effects in the model.

Response	Dairy A					Dairy B				
	Control		Fish meal		SE	Control		Fish meal		SE
	(no.)	\bar{X}	(no.)	\bar{X}		(no.)	\bar{X}	(no.)	\bar{X}	
Cows	166		175			146		154		
Estruses before AI, no.	166	0.53	174	0.43	0.05		NE ¹		NE	
Cows detected in estrus before 48 d PP, ² %	166	40.9	176	42.7	3.7		NE		NE	
Estrus detection rate, ³ %										
First synchronization	166	65.3	175	57.1	4.8	109	50.0	116	51.4	5.6
Second synchronization	57	58.7	76	53.3	6.7	34	49.2	47	71.6	8.6
Pregnancy rate, ³ %										
First synchronization	166	28.7	175	20.7	4.2	109	14.4	116	18.4	4.0
Second synchronization	56	23.5	76	24.1	6.0	40	10.2	58	18.4	4.9
Conception rate, ³ %										
First synchronization	109	40.1	99	32.9	6.6	56	29.1	50	37.5 ⁴	7.9
Second synchronization	31	36.4	39	38.5	9.8	17	9.0	29	24.5	11.1
Overall pregnancy rate to 120 d PP, ⁵ %	161	65.4	174	60.2	3.7	144	31.9	150	41.3	4.0
Overall conception rate to 120 d PP, %	155	66.5	159	70.1	5.9	133	33.4	141	41.3	6.4
Conception rate at first AI, ⁶ %	159	42.3	160	40.9	6.4	133	19.7	150	22.1	5.0
Days open to 120 d PP (for cows that conceived), d	106	78.0	105	79.5	2.7	47	74.0	62	77.2	4.0
AI per Conception to 120 d PP, no.	106	1.5	105	1.4	0.10	46	1.4	62	1.4	0.11
Time to first AI, ⁷ d	155	68.0	159	69.8	1.8	133	64.8	141	64.6	1.4
Interval from PGF _{2α} to AI, d	103	3.2	97	3.2	0.1	88	3.1	87	3.1	0.1
Progesterone concentration, ng/ml										
51 d PP	162	5.6	170	5.4	0.35	137	4.5	141	4.7	0.38
58 d PP	153	4.5	166	4.4	0.29	136	5.2	147	4.5	0.34
65 d PP ⁸	58	2.3	77	1.9	0.27	42	1.5	53	2.6	0.30
72 d PP	56	5.9	70	5.8	0.49	38	6.4	55	6.6	0.68
2 d Post-PGF _{2α}		NE		NE		25	0.57	31	1.26	0.30

¹Not estimated.

²Postpartum.

³Cows undergoing timed AI at dairy B were not represented in these analyses because these cows were synchronized only once.

⁴Column means within dairy B differ in response ($P < 0.07$).

⁵Interaction of diet and dairy ($P < 0.06$).

⁶Includes synchronized and unsynchronized AI.

⁷Includes all cows that were inseminated by 120 d PP.

⁸Means within a row for dairy B differ in response ($P < 0.01$).

12.9%, respectively, and for BUN were 8.7 and 1.6%, respectively. Cows were considered to have undergone luteolysis when plasma concentrations of progesterone declined to <1 ng/ml.

Cows were scored for body condition (five-point scale; 1 = thin to 5 = fat) (11) between 0 and 10 d, at 30 ± 3 d, and at 58 ± 3 d PP for cows at dairies A and B. A final score was recorded at 104 ± 1 d PP for cows at dairy A and at 96 ± 2 d PP for cows at dairy B.

Statistical Analyses

Data were analyzed using the GLM procedure of SAS (26). The mathematical model used to analyze rates of estrus detection and pregnancy rates at synchronized estrus for both dairies included effects of dietary treatment, parity, month of synchronization (January to May), and synchrony (first or second). The effect of reproductive strategy was included in the model for dairy B. For analysis of conception rate, the effect of inseminator also was included in the model. For dairy B, which involved 12 inseminators, 8 inseminators were pooled together because each inseminated <4 cows.

Other reproductive responses evaluated included the number of days to first AI (for all cows inseminated by 120 d PP), number of AI per conception, overall pregnancy and conception rates by 120 d PP, and number of days open for cows that conceived by 120 d PP. These analyses examined the effects of diet and, at dairy B, strategy (strategy 1 = breeding at detected estrus, strategy 2 = timed AI). Interactions of dairy and diet were calculated and are reported when significant. In addition, the interval between the injection of $\text{PGF}_{2\alpha}$ at 58 ± 3 d PP and AI within 7 d was compared between dietary groups.

Plasma concentrations of progesterone at the time of the GnRHa and $\text{PGF}_{2\alpha}$ injections were analyzed using the GLM procedure; dietary treatment was included in the model. Chi-square analysis of dietary treatments at dairy B was used to determine whether differences existed in the distribution of 56 cows with plasma concentrations of progesterone that were >1 ng/ml versus those with concentrations of progesterone that were ≤ 1 ng/ml at 48 h after the injection of $\text{PGF}_{2\alpha}$. The analyses of BUN included dietary treatment, parity, and their interactions in the mathematical model using the GLM procedure.

Body condition scores at 0 to 10 d, 30 and 58 d PP, and the final BCS and changes in BCS between calving and 30 d PP, calving and 58 d PP, and 30 to 58 d PP were analyzed using the GLM procedure; dietary treatment, parity (when significant), and synchrony

were the independent variables. In addition to this mathematical model, a separate analysis included only treatment and parity so that cows inseminated by timed AI at dairy B could be included.

Regression analyses (26) were used to evaluate the relationship between plasma concentrations of progesterone or BCS at 58 d PP and detection of estrus, pregnancy rate, and conception rate as dependent variables adjusted for independent variables such as diet, parity, month of synchronization, and inseminator. For analyses of BCS, synchronization was included in the mathematical model; cows synchronized only once were compared with cows that were not detected in estrus and were resynchronized. Regression analyses also were used to examine the relationship between change in BCS and estrus detection, pregnancy rate, and conception rate; dairy and dietary treatment were included as independent variables in the mathematical model. Body condition score was included in separate analyses as a continuous independent variable to examine the association of BCS with the least squares means of milk production for the experimental period. The order of regression for each was tested.

Data for milk production and composition were analyzed using the GLM procedure (26). The mathematical model included diet, cow within diet, parity, DIM, and their interactions. Orthogonal contrasts were used to determine the effect of parity, diet, and interactions of diet and parity. Contrasts for parity were 1) second versus third or greater, 2) third versus fourth or greater, and 3) fourth versus fifth or greater. Analyses were performed that utilized the mature equivalent milk production from previous lactations as a covariable. The response variable was the least squares mean for milk production for the experimental period adjusted for DIM.

RESULTS AND DISCUSSION

Reproductive Responses

Dietary fish meal did not influence the occurrence of spontaneous estrus prior to estrous synchronization. At dairy A, the proportion of cows that were detected in estrus prior to synchronization by 48 d PP averaged $41.8 \pm 3.7\%$ across both dietary treatments (Table 3). The number of detected estruses prior to synchronization also was not affected by diet. Based on the proportion of cows at 51 d PP with plasma concentrations of progesterone that were >1 ng/ml (74.0 and 72.6% at dairy A and 58.9 and 55.7% at dairy B for cows fed the control diet and cows fed fish

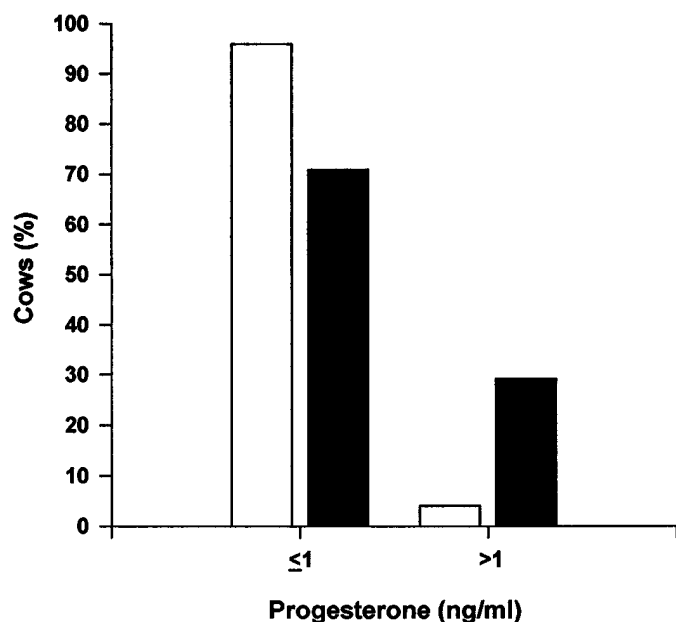


Figure 1. Proportion of cows (percentage) consuming the control diet (□; n = 25) or the diet supplemented with fish meal (■; n = 31) at dairy B at 48 h after injection of PGF_{2α} (58 d postpartum) with plasma concentrations of progesterone that were ≤1 or >1 ng/ml. A greater proportion of cows that consumed the fish meal had concentrations that were >1 ng/ml ($P < 0.025$).

meal, respectively) and the proportion of cows expected to be in the nonluteal phase of the estrous cycle (23.8%), the proportion of cows estimated to be cycling was 97% at dairy A and was 81% at dairy B.

For both dairies, the synchronization program using an injection of GnRHa to recruit a new follicle followed 7 d later by an injection of PGF_{2α} to regress an existing or induced corpus luteum (used for all cows at dairy A and for 75% of cows at dairy B) was effective. In response to the injection of GnRHa at 51 d PP, the proportion of cows with plasma concentrations of progesterone that were >1 ng/ml (78.3 and 82.3% at dairy A and 78.1 and 80.2% at dairy B for cows fed the control diet and cows fed fish meal, respectively) increased such that the proportion of cows estimated to be cycling by 58 d PP was nearly 100% for both dairies A and B. After synchronization was initiated at 51 d PP, 50 to 65% of the cows were detected in estrus (Table 3). At dairy A, the rate of estrus detection at both first and second synchronization was unaffected by dietary treatment (Table 3). The weighted mean response was 59.6%. For dairy B, the rate of estrus detection for cows fed fish meal tended to increase from first to second synchronization (interaction of treatment and synchronization, $P < 0.11$; Table 3). Therefore, approximately 85 and

80% of cows fed the control and fish meal diets, respectively, at dairy A and 74 and 90% of the cows fed the control and fish meal diets, respectively, at dairy B were inseminated between 55 and 78 d PP using the reproductive management system whereby GnRHa and PGF_{2α} were injected, and cows were bred at standing estrus as well as using the timed AI program.

Pregnancy and conception rates at each synchronized estrus were similar between dietary treatments at both dairy farms. However, conception rate at dairy B tended to decline from first to second synchronization ($P < 0.07$; Table 3), likely because fertility was impaired in cows that required a second synchronization. Pregnancy rate by 120 d PP tended to be improved ($P < 0.06$; interaction of diet and dairy) when cows were fed fish meal at dairy B (31.9% vs. 41.3%). Dietary fish meal did not improve fertility at dairy A. Other reproductive responses, including conception rate at first AI, number of days open, number of AI per conception, number of days to first AI, and interval from PGF_{2α} injection to AI, were unaffected by diet at both farms.

Injection of PGF_{2α} initiates regression of the corpus luteum. Induced regression assisted with the final development of the follicle that was recruited after injection of GnRHa. Both EPA and DHA have been shown to inhibit prostaglandin synthesis in the seminal vesicles of rams (10, 29) and can escape microbial biohydrogenation in the rumen (2, 22, 30). Therefore, ingestion of these fatty acids could potentially inhibit prostaglandin synthesis in the lactating dairy cow. If this inhibition were to occur, the dynamics of corpus luteum regression may be altered because uterine endogenous secretion of PGF_{2α} may be reduced and luteal regression would be more dependent on exogenous PGF_{2α}. Indeed, 2 d after injection of PGF_{2α} (58 d PP), the proportion of cows with plasma concentrations of progesterone that were >1 ng/ml was greater when fish meal was fed than when the control diet was fed (29% vs. 4%; $P < 0.025$; Figure 1), suggesting that fish meal altered the dynamics of corpus luteum regression that was induced by injection of PGF_{2α}. In support of this suggestion, cows fed diets supplemented with fish meal that failed to respond to the first synchronization treatment at dairy B had a greater ($P < 0.01$) concentration of progesterone (2.6 vs. 1.5 ± 0.30 ng/ml) 7 d following the luteolytic dose of PGF_{2α} (65 d PP) than did cows that consumed the control diet (Table 3). We assumed that fish meal altered the dynamics of corpus luteum regression but did not block induction of estrus because rates of estrus detection did not differ between diets.

At dairy B, the plasma concentration of progesterone at 58 d PP was related more positively to pregnancy rate at synchronized estrus for cows fed fish meal than for those fed the control diet ($P < 0.025$). Pregnancy rate improved 3.2 percentage units for every 1 ng/ml increase in plasma concentration of progesterone at 58 d PP for cows fed fish meal; the 0.3 percentage unit increase per 1 ng/ml for cows that consumed the control diet [$y_{\text{control}} = 14.71 + 0.34x$; $y_{\text{fish meal}} = -0.37 + 3.20x$, where y = pregnancy rate (percentage) and x = plasma concentration of progesterone (nanograms per milliliter); heterogeneity of regression, $P < 0.002$; $R^2_{\text{model}} = 0.06$; $r^2_{\text{regression}} = 0.05$]. This relationship was not due to a difference in the proportion of anestrus cows, which did not differ between diets at dairy B. Perhaps the inclusion of fish meal in the diet altered the response of the uterus to progesterone produced from the corpus luteum after AI. Pregnancy rate at 120 d PP tended to be greater in cows that consumed fish meal.

The shifting of the reproductive management system at dairy B to timed AI proved acceptable. At dairy B pregnancy rates at the synchronized estrus using timed AI were comparable with those using insemination at standing estrus ($16.2 \pm 2.9\%$ vs. $15.3 \pm 4.6\%$). However, conception rate tended to be lower using timed AI ($33.1 \pm 5.3\%$ vs. $16.3 \pm 8.1\%$; $P < 0.07$) because all cows were inseminated, including cows that would not have responded to synchronization. At dairy B, the number of days to first AI (60.5 ± 1.5 vs. 68.9 ± 1 d; $P < 0.001$) and the interval between injection of $\text{PGF}_{2\alpha}$ and AI (2.9 ± 0.1 vs. 3.2 ± 0.1 d; $P < 0.04$) were reduced using timed AI because all cows were inseminated 3 d after the injection of $\text{PGF}_{2\alpha}$. Conversely, only 50% of cows using strategy 1 were determined to be in estrus by this time and were inseminated 0 to 7 d after $\text{PGF}_{2\alpha}$ injection.

Month of the study influenced rates of estrus detection, conception, and pregnancy at dairy A only. Rate of detection of estrus to synchronized estrus declined ($P < 0.005$) from January ($72.8 \pm 5.6\%$) to April ($46.0 \pm 6.8\%$) perhaps because of a change in environmental temperature, management, or both. Pregnancy and conception rates by 120 d PP declined with month of first synchronization: pregnancy rate was $82.6 \pm 6.2\%$ in January and $48.7 \pm 8.2\%$ in April ($P < 0.001$); conception rate was $86.7 \pm 6.2\%$ in January and $50.8 \pm 8.2\%$ in April ($P < 0.001$). The number of days to first AI increased as month of synchronization progressed at dairy A (66.3 ± 2.4 d in January and 76.0 ± 3.1 d in April; $P < 0.02$), possibly because of fluctuations in management toward the end of the study.

BCS

Dietary effects. Dietary treatment did not affect BCS at any time during the experiment at dairy A (Table 4). Mean BCS of cows at calving was 3.4. Cows lost 0.4 units of BCS by 30 d PP and returned to their starting body condition by 104 d PP (Table 4). At dairy B, initial BCS was 3.4 and 3.2 for cows fed the control or fish meal diets, respectively. Cows fed each diet lost 0.5 units of BCS by 58 d PP. Consumption of fish meal reduced ($P < 0.03$) BCS at 58 d PP by 0.2 units compared with consumption of the control diet (Table 4). Cows at dairy B lost more condition than did cows at dairy A and did not return to their initial body condition by the end of the study as did cows at dairy A. At 96 d PP, cows were still 0.3 to 0.6 units of BCS below their score at calving. Less body condition during breeding might have contributed to the lower pregnancy and conception rates at dairy B.

Influence on reproductive responses. Two populations of cows were evident at dairy A: cows that required only one synchronization to initiate estrous behavior with a greater BCS at 30 ($P < 0.04$), 58 ($P < 0.001$), and 104 d PP ($P < 0.005$) and cows that failed to show signs of estrus at the first synchrony and thus required a second synchronization (Figure 2). A differential relationship between BCS at 58 d PP and estrus detection, pregnancy, and conception rates existed between the two groups of cows. Body condition at 58 d PP was positively ($P < 0.005$) related to the rate of estrus detection for cows synchronized a second time ($y_{\text{synchrony 2}} = -14.1 + 23.8x$, where y = rate of estrus detection and x = BCS; $P < 0.01$; $R^2_{\text{model}} = 0.37$; $r^2_{\text{regression}} = 0.05$). However, BCS had little relationship to a successful first synchrony ($y_{\text{synchrony 1}} = 98.9 + 1.4x$). For every 0.5-unit increase in BCS at 58 d PP for cows synchronized twice, the rate of estrus detection increased more (11.9 percentage units) than for cows synchronized once (0.7 percentage units). A similar positive relationship existed between BCS at 58 d PP and pregnancy rate ($y_{\text{synchrony 2}} = -53.9 + 26.4x$, $y_{\text{synchrony 1}} = 46.7 + 1.4x$; $P < 0.01$; $R^2_{\text{model}} = 0.08$; $r^2_{\text{regression}} = 0.04$) as well as between BCS at 58 d PP and conception rate ($y_{\text{synchrony 2}} = -54.6 + 29.2x$, $y_{\text{synchrony 1}} = 39.0 - 1.5x$; $P < 0.05$; $R^2_{\text{model}} = 0.04$; $r^2_{\text{regression}} = 0.04$) for cows synchronized twice versus those synchronized only once. For every 0.5-unit increase in BCS at 58 d PP for cows synchronized twice, pregnancy rate increased 13.2 percentage units, and conception rate increased 14.6 percentage units. These associations suggest, that among cows that failed to respond to the first synchronization, those with a lower BCS at 58 d PP likely were meeting metabolic

TABLE 4. Mean (\pm SE) body condition scores (BCS) and blood urea nitrogen (BUN) concentrations (milligrams/100 ml of plasma) of cows that consumed the control diet or the diet supplemented with fish meal at two Florida dairy farms. Least squares means are adjusted for appropriate effects in the model.

Response	Dairy A					Dairy B				
	Control		Fish meal			Control		Fish meal		
	(no.)	\bar{X}	(no.)	\bar{X}	SE	(no.)	\bar{X}	(no.)	\bar{X}	SE
BCS										
0 to 10 d PP ¹	69	3.4	81	3.4	0.07	79	3.4	90	3.2	0.07
30 d PP	164	3.0	169	3.0	0.05	79	2.8	90	2.8	0.06
58 d PP ²	142	3.1	158	3.1	0.05	124	2.9	134	2.7	0.06
Final ³	142	3.3	140	3.4	0.06	138	2.8	145	2.9	0.06
BCS Change										
0 to 30 d PP	67	0.39	79	0.35	0.06	50	0.43	62	0.47	0.06
0 to 58 d PP	51	0.39	69	0.29	0.08	55	0.42	67	0.51	0.06
30 to 58 d PP ²	140	0.08	152	0.09	0.04	95	0.01	108	0.11	0.04
BUN										
58 d PP	162	17.2	167	17.3	0.2	124	21.4	134	21.3	0.4
Final concentration ^{3,4}	140	17.0	142	16.4	0.2	138	21.5	145	21.3	0.5

¹Postpartum.

²Means within rows for dairy B differ ($P < 0.03$).

³Final BCS and BUN concentrations were measured at 104 ± 1 d at dairy A and at 96 ± 2 d at dairy B.

⁴Means within rows for dairy A differ ($P < 0.07$).

demands for lactation prior to meeting those for reproduction. Cows with a lower BCS were less likely to express behavioral estrus when synchronized, and perhaps fertility also was impaired.

Conversely, at dairy B, only one population of leaner cows was apparent. The relationships between BCS and estrus detection, pregnancy, or conception rate was not different between synchronies. For all cows at dairy B, for every 0.5-unit increase in BCS at 58 d PP, pregnancy rate increased by 7.0 percentage units [$y = -17.9 + 14.0x$, where y = pregnancy rate (percentage) and x = BCS; $P < 0.03$; $R^2_{\text{model}} = 0.08$; $r^2_{\text{regression}} = 0.03$]. Similarly, BCS at 58 d PP tended to be positively related to rate of estrus detection ($y = 28.1 + 11.0x$; $P < 0.10$; $R^2_{\text{model}} = 0.10$; $r^2_{\text{regression}} = 0.01$) as well as to conception rate ($y = -13.5 + 13.8x$; $P < 0.09$; $R^2_{\text{model}} = 0.07$; $r^2_{\text{regression}} = 0.02$). Cows at dairy B, which produced about 4 kg/d more milk than cows at dairy A, were likely directing metabolic signals that prioritized lactation over reproduction.

Across both dairies, changes in BCS from calving to 30 d PP and from calving to 58 d PP were negatively related to rates of estrus detection, pregnancy, and conception at first synchronization (Table 5). For example, the rate of estrus detection decreased by 18.5 percentage units for every 1.0-unit loss in BCS from calving to 30 d PP. No such relationships were detected for change in body condition between d 30 and 58 PP. Thus, the degree of loss in body condition from calving appears to be associated with reproductive responses.

BUN

At the time of first estrus synchronization, BUN concentrations were similar between cows fed the two experimental diets at dairies A and B (Table 4). At

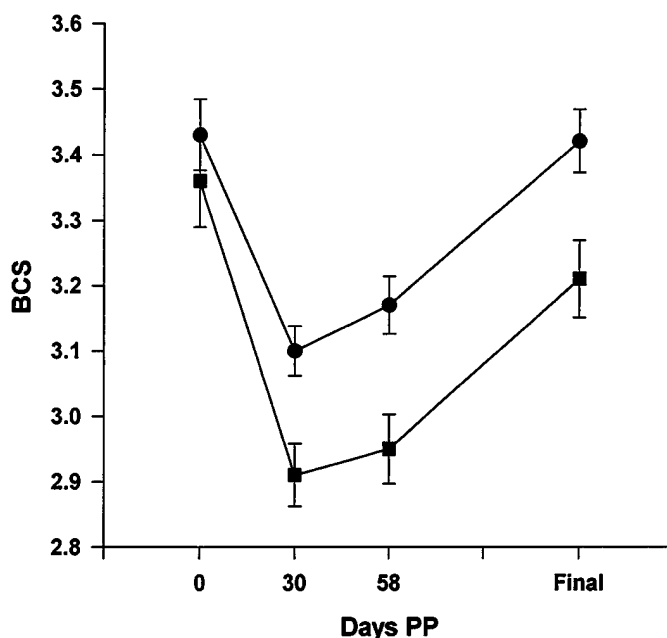


Figure 2. Mean (\pm SE) body condition score (BCS) at 0 to 10 d, 30 \pm 3 d, 58 \pm 3 d, and 104 d postpartum (PP) for cows undergoing one synchronization (\bullet ; $n = 108$) or two synchronizations (\blacksquare ; $n = 133$) at dairy A. The BCS were measured on a five-point scale where 1 = thin to 5 = fat (11).

TABLE 5. Relationship between change in body condition score and rates of estrus detection, pregnancy, and conception across both dairies.

y	x	Equation	R ² _{model} ²	r ² _{regression} ³	P
Estrus detection rate, %	0 to 30 d PP ¹	y = 59.2 - 18.5x	0.11	0.03	0.008
Estrus detection rate, %	0 to 58 d PP	y = 57.7 - 18.0x	0.09	0.03	0.009
Pregnancy rate, %	0 to 30 d PP	y = 27.0 - 16.8x	0.08	0.03	0.005
Pregnancy rate, %	0 to 58 d PP	y = 25.9 - 15.6x	0.08	0.04	0.005
Conception rate, %	0 to 30 d PP	y = 38.5 - 19.7x	0.04	0.04	0.02
Conception rate, %	0 to 58 d PP	y = 35.3 - 23.2x	0.07	0.06	0.004

¹Postpartum.

²Proportion of the total sums of squares attributable to all of the variables in the statistical model.

³Proportion of the total sums of squares attributable to the regression equation.

104 d PP, concentrations of BUN at dairy A tended to be lower in cows that consumed fish meal than those in cows that consumed the control diet (16.4 vs. 17.0 ± 0.2 mg/100 ml; *P* < 0.07); however, values were similar between dietary treatments at dairy B. Cows at dairy B appeared to have consistently greater concentrations of BUN than did cows at dairy A (21.3 vs. 17.2 mg/100 ml), perhaps because of a greater concentration of CP and soluble protein in the diets fed at dairy B (Table 1).

Mean BUN concentrations at 104 or 96 d PP at dairies A or B, respectively, were not different between pregnant and nonpregnant cows (17.3 ± 0.19 vs. 17.4 ± 0.25 mg/100 ml at dairy A and 21.2 ± 0.27 vs. 21.5 ± 0.34 mg/100 ml at dairy B; *P* > 0.10). Similarly, regression analyses indicated that BUN at 58 and 120 d PP was unrelated to pregnancy or conception rates at either dairies A or B. This result differs from the results of Canfield et al. (8), who reported that plasma urea nitrogen concentrations were lower in cows that conceived to the first AI than those in cows that did not conceive to first AI. Also, Butler et al. (7) found that urea nitrogen concentrations were lower in milk from cows that conceived than in milk from cows that did not conceive during a 5-d period after AI. Although no differences were detected within a dairy, the apparently greater concentration of BUN in cows at dairy B might have contributed to a lower overall conception rate by 120 d PP relative to that of cows at dairy A. Alternatively, higher BUN, management, greater milk production, and lower BCS collectively might have contributed to the poorer reproductive responses observed at dairy B.

Feed Intake and Milk Production and Composition

Feed intake was 23.6 and 24.0 kg/d of DM at dairy A and 24.7 and 25.0 kg/d of DM at dairy B for cows fed control diets and diets supplemented with fish

meal, respectively. Because cows were fed in groups and not individually, DMI data could not be analyzed statistically.

Mean milk production was 42.3 and 46.4 kg/d per cow at dairies A and B, respectively, over the duration of the experiment (Table 6). As expected, cows of different parities differed in milk production (*P* < 0.002); cows in their second parity produced less milk. Diet influenced milk production of cows at dairy B, but an interaction of diet and parity was detected (*P* < 0.03). When fish meal was included in the diet, cows in their second parity produced 2.3 kg/d more milk (interaction of diet and second vs. third or greater parities, *P* < 0.01) and cows in their fourth parity tended to produce 3.1 kg/d less milk (interaction of diet and fourth vs. fifth or greater parities, *P* < 0.07) than did cows fed the control diet (Table 6). Conversely, diet did not influence milk production at dairy A. Fewer estimates of milk production at dairy A than at dairy B might have reduced experimental sensitivity to detect an effect of fish meal.

When milk production was adjusted using mature equivalent milk weight as a covariable, dietary effects on milk production remained the same (data not shown). Milk production was unaffected by dietary fish meal at dairy A, but milk production of second parity cows was stimulated by 2.3 kg/d per cow at dairy B (interaction of diet and second vs. third or greater parities, *P* < 0.02).

Reports in the literature on the effects of replacing soybean meal with fish meal in the diet on milk production have not been consistent. Several studies reported no effect of fish meal on milk production in early lactation (5, 28, 32, 34). However, Carroll et al. (9) reported greater milk production by cows fed fish meal during the first 6 wk PP. Cows fed low concentrate diets supplemented with fish meal produced 1.5 kg/d more milk than did cows fed low concentrate diets supplemented with ground nut meal in early lactation (22). However, there was no positive

TABLE 6. Effect of dietary fish meal on uncorrected milk production (kilograms per day) by lactating dairy cows at two Florida dairy farms.

Parity	Dairy A					Dairy B				
	Control		Fish meal		SE	Control		Fish meal		SE
	(no.)	\bar{X}	(no.)	\bar{X}		(no.)	\bar{X}	(no.)	\bar{X}	
All ^{1,2}	162	42.3	174	42.5	1.2	147	46.4	156	46.2	1.1
Second ³	55	41.9	85	40.6	1.6	61	42.6	54	44.9	1.6
Third	46	44.1	28	44.1	2.3	34	47.0	50	46.3	2.0
Fourth ⁴	26	42.8	30	44.1	2.7	24	49.9	25	46.8	2.5
Fifth or greater	35	40.6	31	41.0	2.4	28	46.0	27	46.7	2.5

¹Means within a column differ according to parity (dairy A, $P < 0.002$; dairy B, $P < 0.001$).

²Interaction of diet and parity for dairy B ($P < 0.03$).

³Interaction of diet and second versus third or greater parities ($P < 0.01$).

⁴Interaction of diet and fourth versus fifth or greater parities ($P < 0.07$).

response when fish meal was supplemented to a high concentrate diet. When corn silage was the main dietary forage source, milk production of cows fed diets supplemented with fish meal improved by 1.6 kg/d per cow (24). The current study demonstrates that productive responses caused by dietary fish meal might be associated with parity of the cow.

Only at dairy B was milk analyzed for fat, protein, and SCC content. Diet did not influence milk fat content, although cows in each parity, with the exception of cows in their fourth parity, produced milk with a numerically greater fat percentage when fed fish meal (Table 7). This effect contributed to improved daily production of fat by the oldest cows compared with those in their fourth lactation (interaction of diet and fourth vs. fifth or greater parities, $P < 0.01$; Table 7). This same interaction was true for produc-

tion of 4% FCM ($P < 0.01$; Table 8). Consequently, only cows in their fourth parity failed to produce more 4% FCM when fed fish meal (interaction of diet and fourth vs. fifth or greater parities, $P < 0.01$; Table 8). Atwal and Erfle (3) observed a similar increase in milk fat percentage and production when fish meal replaced soybean meal in a diet based on corn silage and alfalfa silage. In contrast, others reported a decrease in milk fat production (34) and percentage (30, 32, 34) when fish meal was included in a diet based on corn silage for cows in early lactation. The effect of dietary fish meal on milk fat content may be pH dependent. Using continuous cultures, Hoover et al. (13) reported that partial replacement of soybean meal with fish meal and urea reduced the ratio of acetate to propionate when pH was maintained at 6.2, but the ratio was improved by fish meal when the pH

TABLE 7. Effect of dietary fish meal on milk fat percentage and production by lactating dairy cows at dairy B.

Measurement	Control		Fish meal		SE
	(no.)	\bar{X}	(no.)	\bar{X}	
Milk fat, %					
All parities	133	3.19	149	3.27	0.05
Second parity	58	3.20	51	3.28	0.08
Third parity	28	3.20	48	3.40	0.09
Fourth parity	23	3.25	24	3.12	0.12
Fifth or greater parities	24	3.10	26	3.28	0.12
Milk fat, kg/d					
All parities ^{1,2}	133	1.52	148	1.57	0.06
Second parity	58	1.41	50	1.52	0.09
Third parity	28	1.52	48	1.62	0.11
Fourth parity ³	23	1.68	24	1.52	0.15
Fifth or greater parities	24	1.47	26	1.63	0.15

¹Parity ($P < 0.04$).

²Interaction of diet and parity ($P < 0.06$).

³Interaction of diet and fourth versus fifth or greater parities ($P < 0.01$).

TABLE 8. Effect of dietary fish meal on 4% FCM production (kilograms per day) by lactating dairy cows at dairy B.

Parity	Control		Fish meal		
	(no.)	\bar{X}	(no.)	\bar{X}	SE
All ¹	133	41.9	148	42.9	1.3
Second	58	38.7	50	41.5	2.0
Third	28	42.0	48	43.3	2.3
Fourth ²	23	45.9	24	42.5	3.0
Fifth or greater	24	41.3	26	44.4	3.1

¹Parity ($P < 0.002$).

²Interaction of diet and fourth versus fifth or greater parities ($P < 0.01$).

was <6.0. When fish meal was fed at 0, 2.6, 5.2, and 7.8% of dietary DM, milk fat content decreased linearly without a change in the proportion of ruminal VFA (30).

Dietary fish meal increased the milk protein concentration of cows in their fourth parity, but not that of cows in later parities (interaction of diet and fourth vs. fifth or greater parities, $P < 0.04$; Table 9). Production of milk protein tended to increase ($P < 0.11$) across all parities, particularly for second parity cows (interaction of diet and second vs. third or greater parities, $P < 0.04$; Table 9) when fish meal replaced corn gluten, blood, and meat and bone meals in the diet. Similarly, Mantysaari et al. (19) demonstrated an increase in milk protein percentage in primiparous cows that consumed fish meal compared with cows that consumed a protein supplement containing a mixture of meat and bone, meat, poultry by-

product, blood, and hydrolyzed feather meals. Other studies report no change in milk protein percentage or production in cows that consumed fish meals compared with cows that consumed soybean meal supplement (3, 30, 32, 34).

Differences in production responses between dairies in response to fish meal might have been caused by differences in milk production (10% more milk was produced at dairy B, resulting in a greater amino acid demand), the failure of cows to regain lost body condition at the end of the study at dairy B (resulting in a greater reliance on dietary amino acids and less reliance on tissue amino acids), or the replacement of a low lysine protein source (corn gluten meal) with a high lysine protein source (fish meal) at dairy B.

Somatic cell scores were greater ($P < 0.01$) for cows that consumed fish meal (3.54 ± 0.17) than for

TABLE 9. Effect of dietary fish meal on milk protein percentage and production by lactating dairy cows at dairy B.

Measurement	Control		Fish meal		
	(no.)	\bar{X}	(no.)	\bar{X}	SE
Milk protein, %					
All parities ¹	133	2.80	149	2.85	0.02
Second parity	58	2.83	51	2.88	0.03
Third parity	28	2.87	48	2.87	0.04
Fourth parity ²	23	2.73	24	2.88	0.05
Fifth or greater parities	24	2.78	26	2.76	0.05
Milk protein, kg/d					
All parities ^{3,4}	133	1.33	148	1.37	0.04
Second parity ⁵	58	1.24	50	1.34	0.05
Third parity	28	1.37	48	1.36	0.06
Fourth parity	23	1.40	24	1.41	0.08
Fifth or greater parities	24	1.33	26	1.36	0.08

¹Parity ($P < 0.05$).

²Interaction of diet and fourth versus fifth or greater parities ($P < 0.04$).

³Control diet versus fish meal ($P < 0.11$).

⁴Parity ($P < 0.001$).

⁵Interaction of diet and second versus third or greater parities ($P < 0.04$).

cows fed the control diet (2.88 ± 0.19). A score of 3.58 represents an SCC of 150,000, and a score of 2.85 represents an SCC of 90,000. Both scores indicate that milk was of high quality.

CONCLUSIONS

Replacement of typical ruminally undegradable protein sources with a ruminant grade Menhaden fish meal, fed at approximately 0.7 kg/d of DM, tended to result in an improved pregnancy rate by 120 d PP at dairy B. Inclusion of fish meal in the diet resulted in altered dynamics of the regression of the corpus luteum. A greater proportion of cows had elevated plasma concentrations of progesterone at 48 h after injection of PGF_{2α}. The EPA and DHA might have attenuated endometrial synthesis of PGF_{2α} and reduced the efficiency of corpus luteum regression.

Lowered fertility at dairy B might have resulted from greater duration of lower body condition. Body condition of cows synchronized twice at dairy A was related positively to rates of estrus detection, pregnancy, and conception. Body condition of all cows at dairy B was related positively to pregnancy rate.

Cows in certain parities fed fish meal at dairy B produced a greater amount of milk, milk fat, FCM, and protein daily over the period of study. However, fish meal provided no benefit to milk production at dairy A; milk composition was not determined at that farm.

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